Study of the effector mechanism involved in the production of haemorrhagic necrosis of the small intestine in rat passive anaphylaxis

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1 The effector mechanism of intestinal necrosis in rat anaphylaxis was studied following several complementary approaches: (i) the use of monoclonal antibodies (mAb) belonging to different classes (IgG1, IgG2b and IgE anti-DNP), (ii) the assay of mediators, and (iii) the use of pharmacological tools.

2 Lethality and haemorrhagic necrosis of the small intestine were observed in IgE-sensitized rats, whereas IgG mAb produced milder physiological disturbances.

3 Inhibition of leukotriene biosynthesis reduced the drop of systemic blood pressure (BP) and the extent of protein-rich plasma exudation but it did not influence the haemorrhagic component of intestinal necrosis.

4 The antihistamine, pyrilamine, partially diminished the haemorrhagic component of the intestinal necrosis.

5 The involvement of mediators related to platelet-activating factor (PAF) was studied by examining the pharmacological effects of these autacoids and of PAF-receptor antagonists (PCA4248, UR12460 and BB823). PAF induced intestinal lesions similar to those observed in IgE-sensitized rats and PAF-receptor antagonists markedly decreased haemorrhage in IgE-sensitized rats.

6 PAF levels were transiently increased after dinitrophenol (DNP)-bovine serum albumin (BSA) challenge in the small intestine of IgE-sensitized rats.

7 These data stress differences in the outcome of anaphylaxis related to the type of receptors for the Fc portion of immunoglobulins that are involved. IgE is the antibody class that elicits the most severe response due to the activation of mast cells via FcεRI (surface receptors that bind IgE antibodies with high affinity), and the only one able to produce intestinal haemorrhagic necrosis.

8 The mast-cell-derived mediators PAF/acetyl-PAF and histamine, most probably associated with tumour necrosis factor α/cachectin (TNF-α), seem to play a central role in the production of the vascular changes required for the extravasation of erythrocytes in the small intestine mucosa.

Keywords: Anaphylaxis; endotoxin; histamine; IgE; ischaemia; leukotrienes; PAF; reversed passive Arthus reaction; shock; tumour necrosis factor

Introduction

The analysis of the cascade of chemical mediators that is triggered following antigen-antibody interaction in anaphylactoid reactions may be hampered by the diversity of potential effector mechanisms, e.g., mediators released by mononuclear phagocytes are important effectors of the vascular disturbances produced by soluble immune complexes and IgG-aggregates (Sánchez Crespo *et al.*, 1985; Doeber *et al.*, 1986; Fernández-Gallardo *et al.*, 1988), and complement-derived anaphylatoxins amplify cardiac anaphylaxis (del Balzo *et al.*, 1989) and activate platelets in the anaphylactoid syndromes elicited by either soluble aggregates of IgG or endotoxin (Fernández-Gallardo *et al.*, 1989; Smedegård *et al.*, 1989). Neither these findings nor the demonstration that mast cells are not required for the cardiopulmonary changes associated with certain anaphylactic reactions (Takeishi *et al.*, 1991) reduce the central role of this cell type in IgE anaphylaxis, that has been emphasized by studies on genetically mast cell-deficient mice. These animals exhibit limited cardiopulmonary changes after challenge with anti-IgE (Martin *et al.*, 1989), as well as significantly decreased neutrophil accumulation, oedema and haemorrhage in the reversed Arthus reaction as compared to congenic controls (Zhang *et al.*, 1991). It has been established that the gut is the main organ of shock in rat anaphylaxis (Sanyal & West, 1958; Fell *et al.*,

1961; Miller *et al.*, 1983). In keeping with this idea, intestinal mast cells are the major source of mast-cell protease II (King *et al.*, 1986) and leukotrienes (Moqbel *et al.*, 1986) in *Nippostrongylus brasiliensis*-primed rats subjected to i.v. challenge of antigen. Moreover, we have reported high amounts of leukotrienes and detectable amounts of platelet activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) in the peritoneal cavity of rats with intestinal necrosis after IgE anaphylaxis (Fernández-Gallardo *et al.*, 1992). The development of hybridoma cell lines (Liu *et al.*, 1980) that produce dinitrophenyl (DNP)-specific murine IgE, IgG1 and IgG2b has made it possible to obtain large amounts of a single class of monoclonal antibodies (mAb) to be used in rats. The use of IgE mAb permits a passive sensitization of circulating basophils and tissue mast cells that spares activation of the complement system; although the activation of mononuclear phagocytes through FcεRII/CD23 may occur (Capron *et al.*, 1977; Rouzer *et al.*, 1982; Wei *et al.*, 1986). Alternatively, the use of either IgG1 or IgG2b allows activation of mononuclear phagocytes through FcγRII (surface receptors that bind IgG1 and IgG2b antibodies), whereas these antibodies show limited ability to activate serosal mast cells (Katz *et al.*, 1992; Pellón *et al.*, 1993). The purpose of this study was to determine the array of chemical mediators involved in the production of intestinal haemorrhagic necrosis in rat passive anaphylaxis. To overcome the difficulties of the analysis, we have designed an experimental approach

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which includes: (i) the use of monoclonal antibodies of the same specificity but belonging to different classes: (ii) the assay of chemical mediators that *a priori* appeared to be involved, and (iii) the use of pharmacological tools.

Methods

In vivo experimental design

Male Sprague-Dawley rats of about 200 g body weight were passively sensitized with mAb IgE by i.p. administration in 0.2 ml of a phosphate-buffered isotonic saline solution, pH 7.4, and used for experiments within a period of 24 h (Leng *et al.*, 1988). The solution injected contained, unless otherwise stated, about 3 mg of total protein as assessed by the method of Bradford (1976). After testing several schedules, including the i.p. route, rats sensitized with either IgG1 or IgG2b showed maximal response when the antibody was administered i.v. 1–2 h before i.v. challenge with DNP-BSA. Rats were anaesthetized by i.p. injection of Nembutal at a dose of 50 mg kg⁻¹. A femoral artery was cannulated for mean BP recording. A jugular vein was used for infusion of drugs and collection of blood samples. The experimental protocol was approved by Institutional Review Board. Platelets were counted in blood samples of 44.5 µl anticoagulated with EDTA-K₂ using a Coulter counter.

Evaluation of protein-rich plasma extravasation

Vascular permeability changes were evaluated by the Evans blue (EB) extravasation method, as described by Jancar *et al.* (1991). The EB was injected into the jugular vein together with DNP-BSA, and blood samples were taken at different times to assay the concentration of the dye in plasma. Rats were exsanguinated 15 min later, except when otherwise stated. The peritoneal cavity was washed with 5 ml of saline solution, and the pancreas, duodenum, jejunum-ileum, and thymus, dissected, weighed, and a fragment of each organ put in formamide (4 ml g⁻¹ wet tissue at 20°C for 24 h) to extract the EB. The concentration of EB was determined by spectrophotometry at a wavelength of 620 nm. The results were plotted on a standard curve of EB, and the content of each sample was expressed as µg g⁻¹ of dry weight tissue or per ml of lavage fluid.

Extraction and quantitation of haemoglobin from intestinal tissue

The concentration of haemoglobin was determined colorimetrically by the cyanomethaemoglobin method (van Kampen & Zijlstra, 1961) using reagents from Sigma, according to the modifications carried out by Tavares de Lima *et al.* (1992). Fragments of the ileal-jejunal portions of the small intestine were excised and minced in 2 ml of potassium cyanide and hexacyanoferrate solution. After 24 h at room temperature in the dark, the tissue was removed, the sample was centrifuged and the optical density of the supernatant was determined spectrophotometrically at 546 nm. The concentration of haemoglobin was calculated by comparison with a standard curve.

PAF assay and characterization

Minced portions of small intestine taken immediately after DNP-BSA challenge were immersed in cold methanol and allowed to stand for 30 min with gentle shaking. After centrifugation at 3,000 g for 20 min, the total lipid was extracted and processed as described (Fernández-Gallardo *et al.*, 1992). Briefly, after evaporation to dryness of the lipid extract, this was dissolved in the medium used as a mobile phase for straight-phase h.p.l.c. (high performance liquid chromatography). This was carried out in a Spheri-5 silica column

using as a mobile phase A[96% propan-2-ol/hexane, 1/1, supplemented with 0.005% acetic acid] and B[4% water] which was linearly increased to 8% during a 15 min period (Blank & Snyder, 1983). The column was eluted for 60 min at 2 ml min⁻¹, and the fractions collected, evaporated to dryness, and resuspended in 100 µl of a HEPES-saline buffered medium supplemented with 0.25% BSA to carry out a bioassay on rabbit platelets according to the procedure described by Henson (1976). The amount of PAF contained in the samples was corrected for recovery and expressed in ng as assessed from comparison with the activity of the standard. The characterization of the platelet-activating activity as PAF was based on its retention time in h.p.l.c., the lability of the activity to previous treatment with phospholipase A₂, resistance to treatment with phospholipase A₁, and blockade of the platelet-activating ability by the PAF-receptor antagonists used throughout the study.

Assay of haemolytic complement

Total haemolytic activity was measured in rat serum by using sheep erythrocytes and haemolytic serum against sheep erythrocytes raised in rabbits (Hudson & Hay, 1976).

Materials and drugs

Monoclonal anti-DNP IgE, IgG1 and IgG2b were obtained from secreting hybridomas grown as ascites tumours in BALB/c mice and were purified by ammonium sulphate precipitation followed by chromatography. The amount of specific antibody was calculated from classical precipitation reactions using antigen and several dilutions of the antibody solution. In the case of IgE anti-DNP the estimated content of mAb was consistent with the measure of protein in the peak eluting with the M_r of IgE in FPLC gel filtration chromatography using a Superose-12 column (Pharmacia-LKB, Uppsala, Sweden). IgG1 was purified by affinity chromatography in a Protein A-Sepharose CL4B column, and IgG2b was purified by anion exchange FPLC in a MonoS HR5/5 column (Pharmacia LKB, Uppsala, Sweden). 1-Hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (PAF), 1-oleoyl-2-acetyl-*sn*-glycero-3-phosphocholine (acyl-PAF), and pyrilamine were from Sigma, Chemical Company St. Louis, MO, U.S.A. DNP-BSA containing 8 mol of DNP per mol of BSA (bovine serum albumin) was prepared according to the method of Eisen (1964). PCA4248 (2-[phenylthio]ethyl 5-methoxycarbonyl-2, 4, 6-trimethyl-1, 4-dihydropyridine-3-carboxylate) was provided by Alter Laboratories, Madrid, Spain, and solubilized as described (Ortega *et al.*, 1990). UR12460 (1-[(N-diphenylmethyl)aminoacetyl]-4-[(2-methyl-3-pyridyl) cyanomethyl] piperazine) was obtained from URI-ACH, S.A., Barcelona, Spain, and solubilized in saline solution (Carceller *et al.*, 1993). BB823 (Whittaker *et al.*, 1992), a gift from British Bio-technology, Oxford, U.K., was solubilized by stoichiometric mixture with 1 N HCl to form the hydrochloride salt, and dilution with saline solution. MK886 (3- [1-(4-chlorobenzyl)-3-*t*-butyl-thio-5-isopropylindol-2-yl]-2, 2-dimethylpropanoic acid, [Gillard *et al.*, 1989]) was a gift from Dr C.B. Pickett, Merck Frosst, Pointe Claire-Dorval, Quebec, Canada. MK886 was solubilized in dimethyl sulphoxide and ethanol and diluted in saline solution for i.v. administration.

Statistically analyses

Data are expressed as the mean ± s.e.mean, or mean and range for data points involving three results. For comparison of two groups of samples normally distributed, Student's two tailed *t* test was used to analyse differences for significance. For comparison of two groups of samples not normally distributed, the Mann-Whitney U test was used. Changes of a variable measured along a period of time were analysed by one way analysis of variance (ANOVA). Statistical proce-

dures were performed using a data base and statistical package (Instat, GraphPAD Software Inc., San Diego, CA, U.S.A.), $P < 0.05$ was considered significant.

Results

Effect of DNP-BSA challenge on systemic blood pressure (BP)

Animals sensitized with either of the mAb showed a drop of mean BP after i.v. challenge with DNP-BSA. The minimal dose of mAb of either IgG subclass that produced significant hypotension was 3 mg kg^{-1} , and optimal effect was observed with 20 mg kg^{-1} . As regards antigen, optimal hypotension in IgG-sensitized rats required 20 mg kg^{-1} , which taking into account the molar ratio of DNP-substitution and the molecular weight of both antigen and mAb, suggests that optimal expression of the pathophysiological response is observed when the antigen/antibody reaction occurs at antigen excess. This was further confirmed by showing significant hypotensive response when rats were challenged with preformed antigen/antibody complexes in excess of antigen (data not shown). The hypotension observed with IgG1 anti-DNP mAb (Figure 1a) was apparently more prolonged than that produced by the same amount of IgG2b anti-DNP antibody (Figure 1b). In IgE-sensitized rats, chal-

lenge with 0.7 mg kg^{-1} DNP-BSA produced a prolonged hypotension which was maintained until the death of the animals (Figure 2). Experiments intended to show pharmacological modulation of the anaphylactic reaction were initially carried out with MK886, an inhibitor of leukotriene biosynthesis, and with PCA4248, a PAF-receptor antagonist. Treatment with either MK886 (5 mg kg^{-1} , i.v., 5 min before antigen challenge) or PCA4248 (2 mg kg^{-1} , i.v., 5 min before antigen challenge), diminished the drop of BP observed in sensitized animals (Figures 1 and 2). The infusion of a bolus injection of PCA4248 after antigen challenge caused a significant increase of mean BP of $28.0 \pm 6.1 \text{ mmHg}$ ($n = 14$, $P < 0.05$). In contrast, MK886 administered after antigen challenge failed to produce this rapid reversal of hypotension, which is consistent with the expected rapidity of effect of a receptor antagonist as compared to an inhibitor of biosynthesis.

Extravasation of EB in peritoneal fluid and selected organs

Accumulation of protein-rich plasma occurred shortly after antigen challenge as judged from both the decay of plasma

Figure 1 Effect of DNP-BSA on mean arterial pressure in IgG1 (a) and IgG2b (b) sensitized rats, and effect of a previous treatment with MK886 (▲) or PCA 4248 (■). Rats were i.v. sensitized with either IgG class mAb at a dose of 20 mg kg^{-1} , 2 h before challenge with DNP-BSA (20 mg kg^{-1} , arrow). MK886 was i.v. administered (5 mg kg^{-1}), 15 min before antigen challenge. PCA 4248 (2 mg kg^{-1}) or vehicle solutions (●) were i.v. administered 5 min before antigen challenge. Data represent mean $\pm$ s.e. mean from 6 to 9 animals in each group. *Indicates $P < 0.05$. For abbreviations in this and subsequent legends, see text.

Figure 2 Effect of DNP-BSA on mean arterial pressure in IgE sensitized rats and effect of a previous treatment with MK886 (▲) or PCA 4248 (■). IgE mAb (15 mg kg^{-1}) was i.p. administered 18 h before i.v. challenge with 0.7 mg kg^{-1} DNP-BSA (arrow). See legend to Figure 1 for additional details. Data represent mean $\pm$ s.e. mean of 12 to 15 animals in each group.

Figure 3 Decay of plasma Evans blue dye (EB) upon DNP-BSA challenge. Rats were sensitized with either IgE mAb (●) or IgG1 mAb (◆). At the times indicated, blood samples of $200 \mu\text{l}$ were collected and the EB content assessed by spectrophotometric assay; (○) indicate a group of non-sensitized animals challenged with 20 mg kg^{-1} DNP-BSA. The arrow indicates the time of challenge with antigen. Doses of antibodies and antigen are those used for the experiments described in Figures 1 and 2. Data represent mean $\pm$ s.e. mean from 6 rats at each point.

EB and its accumulation in tissues. Decay of plasma EB occurred in IgE- and IgG-sensitized rats, but appeared to be longer lasting in the IgE-sensitized rats (Figure 3). The time course of EB accumulation in jejunum-ileum, which is characterized by a rapid increase of dye content up to 10–15 min, and an ensuing drop is shown in Table 1. This can be explained by the accumulation of EB in the peritoneal fluid (Table 2), and in the intestinal lumen because of enhanced mucosal permeability (King & Miller, 1984). Accumulation of EB could be demonstrated in animals sensitized with a dose of IgE mAb as low as 1.5 mg kg⁻¹ (not shown). Study of selected organs indicated an increase of the accumulation of EB in mesentery and pancreas up to 60 min, and a reduc-

Table 1 Accumulation of both Evans blue (EB) and haemoglobin in small intestine of rats sensitized with IgE mAb and challenged with DNP-BSA: time-course

Time (min)	Evans blue dye (µg g ⁻¹ dry tissue)	Haemoglobin (mg g ⁻¹ dry tissue)
0	99 (87–116)	33 (31–37)
2	201 (164–269)	89 (70–118)
5	295 (282–317)	93 (80–116)
15	321 (288–361)	80 (72–88)
30	188 (179–196)	78 (67–91)
60	151 (141–162)	85 (74–95)

Rats sensitized with 15 mg kg⁻¹ IgE anti-DNP and challenged with 0.7 mg kg⁻¹ DNP-BSA were killed at the times indicated and portions of jejunum excised for assay of EB content and haemoglobin accumulation. Data represent mean and range of three animals at each time. EB content and haemoglobin accumulation in rats killed 60 min after challenge with vehicle were similar to those assayed at time 0.

Table 2 Accumulation of Evans blue (EB) in the peritoneum of IgE-sensitized rats: time-course

Time (min)	Evans blue dye (µg ml ⁻¹)	
	Antigen challenge	Vehicle challenge
0	0.9 (0–2)	0.9 (0–2)
2	1.1 (0–2)	0.9 (0–2)
5	3 (2–4)	0.9 (1–2)
15	9 (7–12)	–
30	11 (7–14)	1 (1–2)
60	20 (16–22)	1.5 (1–3)

Rats were sensitized with 15 mg kg⁻¹ IgE mAb and challenged with either antigen or vehicle. At the times indicated the EB in the peritoneal fluid was assayed. Data represented mean and range of three animals at each time.

Table 3 Accumulation of Evans blue (EB) dye in selected tissues in IgE-sensitized animals: time-course

Time (min)	Evans blue dye (µg g ⁻¹ dry tissue)		
	Mesentery	Pancreas	Thymus
0	10 (9–12)	52 (40–70)	26 (24–27)
2	22 (11–33)	69 (36–119)	29 (20–41)
5	44 (26–55)	116 (105–129)	160 (145–175)
15	66 (38–90)	228 (179–281)	280 (230–330)
30	78 (58–98)	–	120 (86–157)
60	103 (83–122)	260 (230–295)	121 (102–140)

Data represent mean and range values of groups of three animals sensitized with 15 mg kg⁻¹ of IgE mAb killed after challenge with DNP-BSA at the times indicated. Accumulation of EB in the organs of IgE-sensitized rats killed 60 min after challenge with vehicle was 18 (15–23) in the mesentery, 67 (49–81) in the pancreas, and 36 (30–41) in the thymus (mean and range in µg g⁻¹ dry tissue).

tion after 15 min in thymus (Table 3). To obtain further insight on the chemical mediation of EB accumulation, experiments were carried out with MK886, the antihistamine, pyrilamine, and PAF receptor antagonists (PCA4248, UR-12460 and BB823). EB accumulation was partially inhibited by MK886 at a dose of 5 mg kg⁻¹, i.v., whereas pyrilamine and UR12460 were ineffective (Figure 4 and solid columns in Figure 5).

Small intestine necrosis produced by DNP-BSA

Histological examination of jejunal-ileal portions of small intestine from DNP-BSA challenged rats showed a normal appearance in 8 out of 12 IgG-sensitized rats, and mild necrotic changes in the remainder animals. IgG1 and IgG2b-sensitized rats presented the same histological pattern (not shown). In contrast the lesions were very marked in IgE-sensitized rats. The prominence of haemorrhagic changes prompted us to use Drabkin's cyanomethaemoglobin assay as a quantitative index of the severity of the lesions. The haemoglobin content in jejunum-ileum of IgE-sensitized rats increased very rapidly after antigen challenge, since maximal levels were observed at 2–5 min and remained without significant reduction up to 60 min (Table 1). This pattern of intestinal changes induced us to carry out fixed-time experiments at 15 min after antigen challenge, since at that time both haemorrhage and EB accumulation showed maximal values. To assess the dependency on the dose of antibody, rats were sensitized with IgE mAb in the range 1.5–30 mg kg⁻¹, surprisingly, both EB accumulation and haemorrhagic necrosis were observed even with the lowest dose. Figure 5 shows the effect of several pharmacological treatments on accumulation of both haemoglobin and EB. MK886 at 5 mg kg⁻¹, given i.v. 30 min prior to antigen challenge showed no significant effect on haemoglobin accumulation in the jejunal-ileal portions, suggesting no effect of 5-lipoxygenase products on the pathogenesis of haemorrhagic changes. In contrast, pyrilamine-treated rats showed a significant reduction of haemoglobin accumulation (67.2 ± 4.8 mg g⁻¹ dry tissue, *n* = 9). A set of three PAF-

Figure 4 Effect of pharmacological treatments on Evans blue dye (EB) accumulation in pancreas and thymus. Animals were killed 15 min after antigen challenge. Open columns indicate control non-sensitized rats (*n* = 10). Closed columns indicate rats sensitized with 15 mg kg⁻¹ IgE mAb (*n* = 12). Cross-hatched columns indicate rats treated with 6 mg kg⁻¹ pyrilamine (*n* = 9). Hatched columns indicate rats treated with i.v. UR12460, 5 mg kg⁻¹ (*n* = 8). Horizontally lined columns indicate rats treated with 5 mg kg⁻¹, i.v. MK886 (*n* = 7). *Indicates *P* < 0.05 (Student's *t* unpaired two-tailed test).

Figure 5 Effect of pharmacological treatments on accumulation of both Evans blue dye (EB) and haemoglobin in the small intestine of IgE-sensitized rats upon antigen challenge. Drugs were administered i.v. prior to antigen challenge at the doses indicated. Figures in parentheses indicate the number of animals in each group. The line at the base of the columns indicates mean control values obtained in 10 IgE-sensitized rats challenged with vehicle. Open columns indicate haemoglobin accumulation. Solid columns indicate EB accumulation. * $P < 0.05$; ** $P < 0.01$.

Figure 6 Accumulation of both Evans blue dye (EB) and haemoglobin in small intestine of rats challenged with different doses of PAF (a) and acyl-PAF (b). Animals were killed 15 min after i.v. infusion of either PAF or acyl-PAF. (●) indicate EB content and (▲) indicate haemoglobin accumulation. Data represent mean $\pm$ s.e. mean of three animals at each point.

Table 4 Accumulation of Evans blue (EB) dye in selected tissues following i.v. PAF administration: dose-response

PAF ($\mu\text{g kg}^{-1}$)	Evans blue dye ($\mu\text{g g}^{-1}$ dry tissue)		
	Mesentery	Pancreas	Thymus
Vehicle	10 (9–11)	52 (34–70)	26 (24–28)
0.4	30 (25–33)	155 (114–175)	98 (83–115)
0.8	31 (23–42)	263 (137–337)	110 (94–141)
4	44 (44–45)	256 (216–286)	264 (198–303)
7.5	42 (27–56)	191 (157–221)	203 (160–232)
10	12 (7–17)	136 (121–147)	173 (150–199)
20	14 (8–19)	117 (63–147)	161 (94–217)
40	12 (5–18)	96 (84–108)	106 (59–142)

Data represent mean and range values of groups of three animals killed 15 min after i.v. administration of PAF.

receptor antagonists showed a more significant protection from haemorrhagic changes ($55.5 \pm 4.8 \text{ mg g}^{-1}$ dry tissue, $n = 12$, in the case of UR12460-treated rats). Since the experiments with PAF-receptor antagonists suggested a possible involvement of PAF-like compounds in the production of haemorrhage, the effect of both PAF and acyl-PAF were tested. As shown in Figure 6a, i.v. administration of PAF at doses of $20 \mu\text{g kg}^{-1}$ caused accumulation of EB and haemoglobin in jejunum-ileum at amounts comparable to those observed in IgE anaphylaxis. A similar result was obtained with 1-acyl-2-acetyl-*sn*-glycero-3-phosphocholine (acyl-PAF), although the study of dose-dependency exhibited a lower pharmacological potency (Figure 6b). As shown in Table 4, maximal accumulation of EB induced by PAF in mesentery, pancreas and thymus was observed with a dose of $4 \mu\text{g kg}^{-1}$, which is slightly different from the dose-dependency obtained in small intestine, which showed maximal responses at $40 \mu\text{g kg}^{-1}$ (Figure 6a).

Assay of PAF and complement consumption

Elevation of PAF levels above those detected in non-challenged rats could be detected only at 30 s and 2 min after

DNP-BSA challenge, whereas they returned to near basal levels 5 min thereafter (Figure 7). Significant complement consumption was observed in rats sensitized with IgE, but not in those sensitized with IgG1 or IgG2b, as judged from the measure of CH_{50} at different times after DNP-BSA challenge. As shown in Figure 8, IgE-sensitized animals showed a drop from 103 ± 13 to 67 ± 12 CH_{50} u ml^{-1} , whereas IgG2b-sensitized animals only showed a drop from 110 ± 4 to 89 ± 17 (Table 5). Platelet count was influenced by antigen challenge in IgG2b sensitized rats ($803,444 \pm 67,550$ cells μl^{-1} before antigen challenge, versus $604,714 \pm 40,696$ cells μl^{-1} 15 min thereafter); however, these changes were nonsignificant (one-way ANOVA).

Discussion

Acute inflammation is characterized by oedema involving extravasation of plasma proteins and cellular infiltration, and only in some circumstances haemorrhage and necrosis occur. This may be the result of complement-dependent neutrophil-mediated injury as in the Arthus reaction (Arthus, 1903), but it may also develop because of an enhanced expression of endothelial-leukocyte adhesion, molecules triggered by cytokines as in the Sanarelli-Schwartzman reaction (Movat *et al.*, 1987). Since these reactions show mechanistic differences with immediate hypersensitivity reactions, it seems of interest to delineate the effector mechanism of intestinal necrosis in

anaphylaxis. It is not surprising that haemorrhage and exudation show different pharmacological modulation, because exudation commonly occurs without bleeding, and is impeded by severe vasoconstriction and stasis (Leng *et al.*, 1988; Ramos *et al.*, 1992). In this regard the intravenous injection of PAF into rats produced an accumulation of EB labelled plasma proteins and haemoglobin in the small intestine increasing with increasing doses of PAF up to $40 \mu\text{g kg}^{-1}$. In contrast, accumulation of EB in mesentery, pancreas and thymus reached a maximum at doses of $4 \mu\text{g kg}^{-1}$ and decreased at higher doses. A likely explanation for these findings would be that the rate of extravasation in the small intestine and into the peritoneal cavity at the higher doses of PAF reduced the amount of EB in the blood that was available for accumulation in the other tissues. Alternatively, the haemodynamic changes produced by the highest doses of PAF could influence the distribution of EB.

The results obtained using mAb of different classes indicated that circulatory disturbances can be elicited by activation of either $\text{Fc}\epsilon$ or $\text{Fc}\gamma$ receptors (Ravetch & Kinet, 1991), but the magnitude of the lesions observed in IgE-sensitized rats points to a central role for $\text{Fc}\epsilon\text{RI}$ on mast cells. The rapid appearance of haemorrhage after antigen challenge suggests the involvement of either preformed mediators or secondary mediators rapidly generated upon stimulation of mast cells. Interestingly, the histological study showed strong similarities with the lesions produced by tumour necrosis factor- α /cachectin (TNF- α) (Tracey *et al.*, 1986; Sun & Hsueh, 1988), PAF (González-Crussi *et al.*, 1983) and lipopolysaccharide (LPS) (Hsueh *et al.*, 1986). The potential of acyl-PAF to induce intestinal damage requires more attention: first, this compound is rapidly produced by mast cells upon IgE-dependent activation (Triggiani *et al.*, 1990; 1991; 1992), and also by endothelial cells in response to a variety of autacoids including histamine

Figure 7 PAF content in small intestine samples from IgE-sensitized rats. Animals were killed before or at different times after DNP-BSA challenge (arrow). A portion of the jejunal-ileal portion of the small intestine was excised, minced and immersed in cold methanol as described under Methods. Data represent mean $\pm$ s.e.mean from 4 rats at each time. * $P < 0.05$ (one-way ANOVA).

Figure 8 Complement consumption in IgE-sensitized rats after DNP-BSA challenge. Blood samples were collected at the times indicated. (●) represent sensitized-animals challenged with antigen; (○) indicate non-sensitized rats challenged with antigen. The arrow indicates the time of antigen challenge. Data represent mean $\pm$ s.e. mean of 8 animals at each time.

Table 5 Effect of antigen challenge on residual complement activity and platelet count in IgG1- and IgG2b-sensitized rats

Time (min)	IgG1-sensitized		IgG2b-sensitized		Controls	
	(CH_{50} u ml^{-1})	(Platelets μl^{-1})	(CH_{50} u ml^{-1})	(Platelets μl^{-1})	(CH_{50} u ml^{-1})	(Platelets μl^{-1})
0	114 ± 4	$919,875 \pm 58,672$	110 ± 4	$803,444 \pm 67,550$	110 ± 5	$846,987 \pm 47,231$
5	93 ± 10	$745,625 \pm 57,534$	89 ± 17	$668,625 \pm 64,220$	107 ± 6	$823,654 \pm 42,324$
15	100 ± 10	$717,750 \pm 97,786$	91 ± 14	$605,714 \pm 40,696$	103 ± 5	$815,896 \pm 53,987$
30	102 ± 9	$735,250 \pm 97,383$	91 ± 16	$651,571 \pm 65,303$	105 ± 7	—
60	105 ± 8	—	88 ± 12	—	107 ± 6	$799,564 \pm 65,876$

Data represent mean $\pm$ s.e.mean of 7 to 9 animals in each group. Differences among groups were not significant at any time (ANOVA).

(MacIntyre *et al.*, 1985; Clay *et al.*, 1991); second, the biological actions of acyl-PAF can be blocked by antagonists of the PAF receptor (Smiley *et al.*, 1991); third, acyl-PAF has a reduced activity on the rabbit platelet PAF receptors and this explains why the production of acyl-PAF by either intestinal mucosal mast cells or peritoneal mast cells, which has been mainly assayed with the rabbit platelet bioassay could have been underestimated (Fernández-Gallardo *et al.*, 1992; Hogaboam *et al.*, 1993). Moreover, the probable formation of PAF and/or acyl-PAF by cells in close contact with the vessels and the slow kinetics of accumulation of peritoneal exudate might explain why PAF assay in the peritoneal exudate did not reveal the transient accumulation of PAF in the small intestine shown here. Again, the measure of autacoids in the peritoneum should reflect the secretion products of connective tissue mast cells, whereas the intestinal mucosal mast cells could generate a different array of chemical mediators.

The concurrent involvement of mast cell-derived TNF- α /cachectin in intestinal haemorrhagic necrosis seems likely since mast cells store TNF- α in their granules (Gordon & Galli, 1990), and also because of the ability of TNF- α to induce vessel damage and haemorrhagic necrosis, especially when interactions with other products occur, e.g., bacterial components (Rothstein & Schreiber, 1988) complement factor C5 (Rothstein *et al.*, 1988), or other cytokines (Waage & Espevic, 1988). In addition, an effector role of PAF in the production of intestinal necrosis produced by exogenous TNF- α has been reported (Sun & Hsueh, 1988). However, some differences exist between the lesions produced by TNF- α in rats and those observed in the present experiments. First, the rapid appearance of lesions in IgE-sensitized rats as compared to longer periods in TNF- α challenged rats. Second, the invariable occurrence of infarct in the caecum in TNF- α challenged rats, as compared to a preferential location to the jejunum and ileum in the present experiments. Third, the absence in the present study of occlusion of the vessels by platelet and leukocyte thrombi, which can be partially explained by the lack of PAF receptors in rat platelets (Sánchez Crespo *et al.*, 1982; Humphrey *et al.*, 1984). Nevertheless, as we observed involvement of the caecum in rats challenged with exogenous PAF, it is possible

that the different locations of intestinal damage could be explained by the predominant release of endogenous mediators in some intestinal areas, whereas exogenous mediators exert their actions throughout the intestine. The involvement of the complement system in rat anaphylactoid syndromes could be suspected in view of the effect of anaphylatoxins on different models of IgE-dependent reactions and the dependence on complement factors of TNF- α and PAF-induced tissue injury (Rothstein *et al.*, 1988; Sun & Hsueh, 1991); however, the results presented would suggest that complement activation is not a sufficient condition for intestinal injury, since the drop of CH₅₀ residual complement was less prominent than in the case of infusion of soluble aggregates of IgG (Fernández-Gallardo *et al.*, 1989), and under these conditions there is no evidence of haemorrhagic necrosis. Alternatively, complement consumption could be the consequence of tissue injury rather than its cause, since IgE antibodies have no complement-fixing properties. In summary, our data indicate that different classes of mAb trigger different types of anaphylactoid reactions. IgE is the only antibody class that elicits haemorrhagic necrosis of the small intestine. The release of histamine and PAF/acyl-PAF from mast cells seems to play a central role in the production of necrosis of the small intestine, whereas 5-lipoxygenase-derived products seem involved in leakage of protein-rich plasma. Activation of Fc ϵ RII and Fc γ RII receptors leads to a generation of mediators adequate for the production of vascular disturbances, but not at the amount and/or location required for the production of intestinal haemorrhagic necrosis.

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